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Dose-Related Differences in DNA Adduct Levels in Rodent Tissues Following Skin Application of Complex Mixtures from Air Pollution Sources

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16. ABSTRACT

Dose-related differences in the binding of DNA reactive intermediates for three environmentally important complex particulate extracts and a well studied carcinogen Benzo(a)Pyrene (B(a)P) were examined in female C-57 mice following multiple topical treatments ranging from (1-120) mg per mouse. Particulate extracts from coke oven, coal soot and diesel exhaust were selected as model complex mixtures based on short term mutagenicity assays, animal bioassays for carcinogenicity or epidemiological studies where increased incidences of lung cancer in exposed populations were detected. Positive and negative control animals were treated with either 1.2 mg Benzo(a)Pyrene or acetone alone. DNA was isolated from skin, lung and liver DNA 24 hours following the last application and analyzed for DNA adducts using the nuclease P1 version of the ^{32}P-postlabeling assay. Each of the particulate extracts produced distinct patterns of DNA adducts. A broad diagonal radioactive zone (DRZ) presumably representing multiple DNA adducts, was observed for coke oven, coal soot and diesel modified DNA samples. One adduct, common to all three complex mixture modified DNA samples, comigrated with the major B(a)P adduct observed following treatment with B(a)P alone. This adduct was detected in all tissues examined and represented approximately 12-34 percent of the total number of adducts detected within the DRZ for all coke oven and smoky coal exposed tissues (skin, lung and liver). In contrast, this adduct represented 49-69 percent of the total radioactivity quantitated in the lung diesel modified DNA samples. The highest total number of adducts resulted from the metabolism of coke oven extract followed by smoky coal and diesel treatments respectively. A dose-dependent increase in adduct formation was observed for all tissues in the diesel and smoky coal treated mice. Liver and lung DNA adduct levels increased in a dose dependent manner in the coal soot and coke oven treated mice. In contrast, no consistent dose-response relationship was observed in the skin DNA samples isolated from the coke oven modified DNA samples. The percentage of dose administered detected as DNA adducts increased in all tissues as the dose decreased for all three complex mixtures. These data have important implications for low dose risk assessment of these complex mixtures.

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Dose-related differences in DNA adduct levels in rodent tissues following skin application of complex mixtures from air pollution sources

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Dose-related differences in the binding of DNA reactive intermediates for three environmentally important complex mixture particulate extracts and a well-studied carcinogen, benzo[*a*]pyrene (BaP), were examined in female C-57 mice following multiple topical treatments ranging from 1 to 120 mg/mouse. Particulate extracts from coke oven, coal soot and diesel exhaust were selected as model complex mixtures based on short-term mutagenicity assays, animal bioassays for carcinogenicity or epidemiological studies, where increased incidences of lung cancer in exposed populations were detected. Positive and negative control animals were treated with 1.2 mg BaP or acetone respectively. DNA was isolated from skin, lung and liver 24 h following the last application and analyzed for DNA adducts using the nuclease P1 version of the ^{32}P-postlabeling assay. Each of the particulate extracts produced distinct patterns of DNA adducts. A diagonal zone of radioactivity, presumably representing multiple putative DNA adducts, was observed for coke-oven, coal-soot- and diesel-modified DNA samples. One adduct, common to all three complex-mixture-modified DNA samples, co-migrated with the major BaP adduct observed following treatment with BaP alone. Based on the BaP concentration for each of the extracts it seems unlikely that this adduct is derived from BaP alone. It is possible that an adduct is formed with chromatographic properties similar to the major BaP-derived adduct detected in mice treated with BaP alone. This adduct was detected in all tissues examined and represented ~12–34% of the total number of adducts detected within the diagonal radioactive zone for all coke-oven- and coal-soot-exposed tissues (skin, lung and liver). In contrast, this adduct represented 49–67% of the total radioactivity recovered from the diagonal zone of DNA isolated from lungs of animals exposed to diesel extract. The highest total number of adducts resulted from the metabolism of coke oven extract followed by coal soot and diesel treatments respectively. A dose-dependent increase in adduct formation was observed for all tissues in the diesel- and coal-soot-treatment mice. Liver and lung, but not skin, DNA adduct levels increased in a dose-dependent manner in the coke-oven-treated mice. The percentage of dose administered, detected as DNA adducts increased in all tissues as the dose decreased for all three complex mixtures. These data have important implications for risk assessment of these complex mixtures.

Introduction

Polycyclic aromatic hydrocarbons (PAHs*) are metabolized efficiently by mammalian cells to DNA reactive intermediates (1,2). The extent of DNA binding for many PAHs is frequently related to their carcinogenic potency (3). Despite the fact that several complex environmental mixtures contain high levels of PAH constituents, few studies have evaluated the formation of DNA adducts in *in vivo* systems.

^{32}P-postlabeling analysis allows for the detection of low levels of bulky aromatic DNA adducts yet does not require identification of specific chemical constituents to measure the overall level of DNA adducts resulting from exposure of complex mixtures (4,5).

Complex environmental mixtures have been tested in *in vivo* rodent tumor models for the purpose of evaluating the extent of DNA adduct formation and relative tissue distributions of the DNA adducts (6–9). These studies demonstrate that multiple putative DNA adducts are formed resulting from *in vivo* exposure to a wide variety of complex environmental mixtures.

Workers exposed occupationally to coke oven emissions, and women in China exposed to high concentrations of coal soot have been shown to be at increased risk for developing lung cancer (10,11). Several epidemiological studies of diesel emissions show increased lung cancer risk (12,13). Moreover, recent studies indicate that rats chronically exposed to diesel emissions develop lung tumors (14–16). Coke oven, diesel and coal soot emission extracts used in this study also induced tumors after skin application in mice (11,17). In the present study, we have examined DNA adduct levels and profiles in skin, lung and liver DNA following multiple skin treatments with coal soot, diesel or coke oven particulate extracts and quantitated the dose-dependent changes in DNA adduct formation.

Materials and methods

Chemicals

[γ-^{32}P]ATP (3000 Ci/mmol, 10 mCi/ml aqueous solution containing 5 mM 2 mercaptoethanol) was obtained from Amersham, Arlington Heights, IL. Polyethyleneimine (PEI) cellulose TLC plates were prepared as described (4) except that the PEI solution (50% aqueous) was from Aldrich Chemical Co., Milwaukee, WI. Micrococcal nuclease and nuclease P1, were from Sigma Chemical Co. St Louis, MO, calf spleen phosphodiesterase was from Boehringer Mannheim, Indianapolis, IN, T4 polynucleotide kinase was from Pharmacia, Piscataway, NJ. All other chemicals were of analytical grade.

Preparation of samples

Extracts of particulates and associated organics from diesel exhaust and coke oven mains were prepared as previously described (17). Extracts were dried under a stream of nitrogen. The dried residues were stored at −80°C. Extracts of the coal soot were prepared in methylene chloride at a concentration of 14.5 mg/ml. Dosing solutions of the coal soot samples were prepared by drying a desired volume under a stream of nitrogen and redissolving in acetone. Stock solutions of 150 mg/ml of each sample were prepared by adding acetone to the dried residues and sonicating in a sonic bath for 30 s. Dilutions of the stock solutions were prepared in acetone to obtain various treatment doses. A benzo[*a*]pyrene (BaP) solution was also prepared at a concentration of 1.5 mg/ml in acetone. BaP-treated mice served as the positive control.

Animal treatment

Female C-57 mice (6–8 weeks old) weighing ~20 g were utilized for this study. Animals were treated using a modification of the method described by Randerath *et al.* (18). Three days prior to treatment the dorsal side of each animal was shaved.

*Abbreviations: PAH, polycyclic aromatic hydrocarbon; PEI, polyethyleneimine; BaP, benzo[*a*]pyrene; RAL relative adduct labeling.

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Table I. DNA binding capacity[a] of adduct 1 normalized to BaP content from skin and lung DNA

Treatment	BaP[a] μg (20-mg dose)	Adducts/10^8 nucleotide/ μg BaP administered	
		Skin	Lung
Diesel	0.52	5.6	7.7
Coal soot	19.68	0.7	0.4
Coke oven	35.10	1.4	0.6

[a]Relative adduct labeling for adduct 1 (adducts/10^8 nucleotides) divided by the equivalent μg BaP applied as determined by the BaP concentration (at the 20-mg dose) for each of the extracts. RAL are for mean values from two or three determinations from at least two ^{32}P-postlabeling analyses, see Table II.

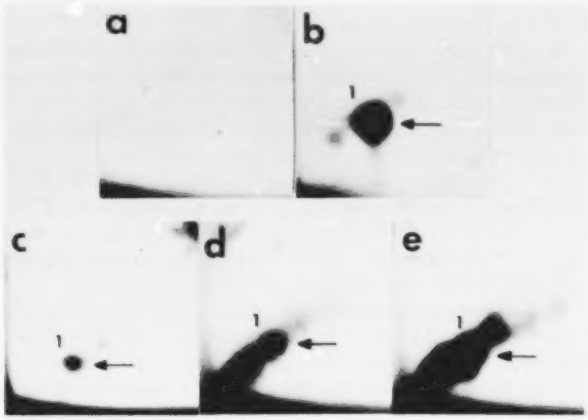

Fig. 1. Autoradiographs of PEI–cellulose plates from ^{32}P-postlabeled skin DNA isolated from mice treated with extracts from complex environmental mixtures. Autoradiography for TLC plates was at −80°C for (**a**) acetone (−) control, 4 h; (**b**) BaP (+) control, 1.2 mg/mouse, 1 h; (**c**) diesel, 20 mg/mouse, 18 h; (**d**) coal soot, 20 mg/mouse, 4 h; and (**e**) coke oven, 20 mg/mouse, 4 h.

Test samples were applied on the shaved areas (4–5 cm^2) using a micropipet. Application of each sample was made in a volume of 200 μl and repeated four times (800 μl total) at 0, 6, 30 and 54 h. Due to limited sample we were only able to use one or two mice per treatment dose. We felt it was more important to obtain data on dose–response differences than to use more animals for a single dose; however, we did address the issue of experimental variability by conducting multiple analyses on samples from each of the one or two rodents per treatment group. Two mice per treatment group at the lower doses and one mouse per treatment group at the highest dose for the coke oven and diesel were dosed as follows: control mice received 200 μl acetone/application, BaP-positive control mice received 200 μl/application of the 1.5 mg/ml stock (1.2 mg/mouse total dose), mice dosed with extracts from the complex mixtures at 120 mg/mouse received 200 μl/application of the 150 mg/ml stock, those dosed at 50 mg/mouse received 200 μl/application of a 62.5 mg/ml solution and those dosed at 20 mg/mouse received 200 μl/application of a 25 mg/ml solution. In addition one group of mice was treated with coal soot extract (2 mice/dose) at 1, 5 and 10 mg/mouse using 200 μl/application at 0, 6, 30 and 54 h as described above. Mice were killed by cervical dislocation 24 h after the last application and skin, lung and liver tissues were collected and stored at −80°C.

DNA extraction

Frozen tissues were thawed and homogenized (20–30%, w/v) in a solution of 1% SDS with 1 mM EDTA. The homogenates were incubated with 1 mg/ml proteinase K for 1 h at 37°C then brought to a 50 mM concentration with Tris–HCl, pH 8.0. DNA was isolated and purified essentially as described previously (19).

^{32}P-postlabeling analysis

DNA (5 μg) was digested to 3′-mononucleotides at 37°C for 3.5 h with micrococcal endonuclease and spleen phosphodiesterase according to the method of Gupta (20). DNA digests were then nuclease P1 treated according to the methods of

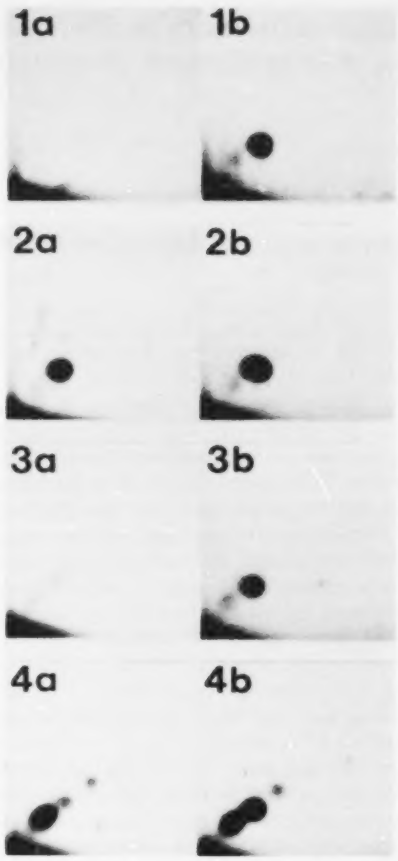

Fig. 2. Co-chromatography of lung DNA isolated from mice treated topically with particulate extracts and spiked with lung DNA from mice treated with BaP. Equal concentrations of lung DNA from mice treated with particulate extracts were mixed with either lung DNA from control mice (non-spiked, **panel a**) or lung DNA from mice treated with BaP (spiked, **panel b**). Autoradiography of the TLC plates was at −80°C for 4 h. 1, Control; 2, diesel; 3, coal soot; 4, coke oven.

Reddy and Randerath (21) with minor modifications as follows. To 12.5 μl of the undiluted DNA digests (5 μg DNA), 1.2 μl nuclease P1 (8.0 μg), 1.8 μl zinc chloride (0.30 mM) and 3.0 μl sodium acetate buffer (0.25 M) pH 5.0 were added. After incubation for 40 min at 37°C, the pH was adjusted by addition of 2.4 μl 0.5 M Tris base pH 9.0. The digest was labeled by adding 7.5 μl of the radioactive mixture containing 50 μCi [γ-^{32}P]ATP, 1.5 μl kinase buffer (300 mM Tris base/100 mM dithiothreitol/10 mM spermidine) and 2.5 U T4 polynucleotide kinase and incubating for 30 min at 37°C. A 26.0-μl aliquot of the total labeled mix was spotted onto the origin on PEI–cellulose TLC plates. The solvent systems and directions for TLC have been previously described (22). To measure the total number of nucleotides, an aliquot containing 0.5 μg of digested DNA sample was diluted ~200-fold with distilled water and an aliquot taken and labeled as above with an equal volume of radioactive mix. The labeled nucleotides were diluted 50-fold with 10 mM Tris–HCl, pH 9.5/5 mM EDTA, and 5 μl were applied to a PEI–cellulose plate which had been soaked in 100 mM ammonium formate, pH 3.5 for 40 min and dried. Chromatography was in one direction with 4.5 M ammonium formate.

Intensifying-screen-enhanced autoradiography at −80°C was used to detect the presence of radiolabeled adducts on the TLC plates. A template was overlayed, marking the boundary of the zone of radioactivity. The area within this boundary was carefully scraped and the radioactivity counted by the Cerenkov assay (23). The same template was used to measure the radioactivity detected within the diagonal zone in the acetone and positive control BaP samples. Individual adducts of interest were also carefully excised and relative adduct level quantitated separately, as shown in Table I.

DNA adduct quantitation

For all DNA adducts, areas of radioactivity were Cerenkov counted in a scintillation counter with 5 ml 95% ethanol/vial. Relative adduct labeling (RAL) values were

Table II. Comparison of RAL[a] in DNA from mouse tissues following exposure to complex environmental mixtures

Treatment	Skin	Lung	Liver
0 mg/mouse			
(−) control	5.0 ± 0.5	2.9 ± 1.0	6.0 ± 2.9
1.2 mg/mouse			
BaP (+ control)	1035.8 ± 399.2 (83)	55.4 ± 12.8 (73)	13.6 ± 4.5 (51)
20 mg/mouse			
Diesel	12.9 ± 2.2 (23)	8.3 ± 1.7 (49)	4.6 ± 1.8 (15)
Coal Soot	75.1 ± 15.8 (19)	32.0 ± 4.8 (23)	11.8 ± 6.2 (15)
Coke Oven	144.5 ± 57.6 (34)	104.1 ± 23.9 (19)	20.8 ± 7.3 (18)
50 mg/mouse			
Diesel	13.9 ± 3.3 (24)	14.3 ± 2.5 (59)	5.6 ± 1.8 (16)
Coal Soot	53.4 ± 11.0 (18)	32.7 ± 7.6 (18)	18.3 ± 4.1 (14)
Coke Oven	210.3 ± 41.6 (32)	111.0 ± 20.3 (17)	30.2 ± 10.6 (18)
120 mg/mouse			
Diesel	19.0 ± 3.6 (19)	32.5 ± 2.8 (67)	7.8 ± 2.8 (21)
Coal Soot	45.2 ± 10.1 (17)	28.5 ± 5.3 (18)	24.9 ± 3.6 (12)
Coke Oven	114.5 ± 14.6 (26)	129.0 ± 18.0 (14)	48.3 ± 10.1 (17)

[a]Relative adduct labeling (adducts/10^8 nucleotides). Values shown represent duplicate or replicate analyses from at least two or three separate ^{32}P-postlabeling analyses and represent mean RAL ± SD for the multiple DNA adducts detected within the boundary of the diagonal zone of radioactivity. Numbers in parentheses represent the RAL for adduct 1 expressed as a percentage of the total radioactivity detected within the diagonal zone.

calculated by dividing the counts per minute detected (in the diagonal zone of radioactivity or individual adducts within this zone) by the counts per minute determined for the total nucleotides, after correction for the background activity, dilution factors and volumes spotted.

Statistical significance is reported in the results on a paired basis using a one-tailed Student's *t*-test with $n-1$ degrees of freedom.

Results

Chromatographic analysis of DNA modified by complex particulate extracts

Using the nuclease P1 version of the ^{32}P-postlabeling assay, a diagonal zone of radioactivity, resulting from the formation of multiple putative DNA adducts was observed for all skin, lung and liver DNA samples following topical application of coke oven, coal soot and diesel particulate extracts in female C-57 mice. Figure 1 shows representative complex mixture skin DNA adduct patterns resulting from the three particulate extract treatments.

One adduct, detected in all complex-mixture-exposed tissues, co-migrated with a major BaP-derived adduct as shown in co-chromatography experiments where lung DNA samples isolated from control and complex-mixture-treated samples were spiked with DNA isolated from the BaP-treated animals (Figure 2). The relative concentration of BaP within each of these mixtures also suggests that this adduct is not derived from BaP alone (Table I). For purposes of clarity, this adduct throughout the text is referred to as adduct 1 (Figure 1). Adduct 1 was detected within the boundary of the zone of radioactivity thus; the RAL for this adduct is represented as a percentage of the total radioactivity detected within the diagonal zone of radioactivity (Table II).

Coke-oven-modified tissues were shown to have significantly higher DNA adduct levels ($P < 0.05$) compared with coal-soot- and diesel-extract-modified DNA tissues at any dose. A histogram at the 20-mg treatment dose is presented in Figure 3 to demonstrate typical differences in adduct concentration. The DNA adduct levels for the remaining doses and tissues are shown in Table II.

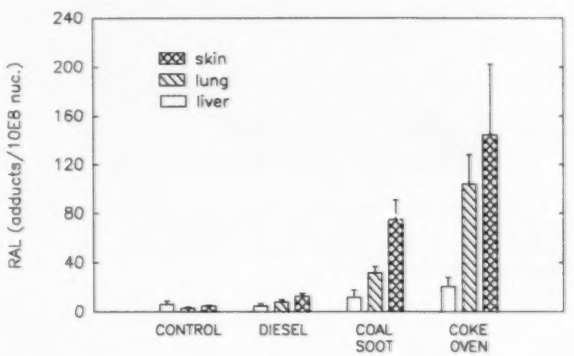

Fig. 3. Histogram of the RAL observed in skin, lung and liver DNA 24 h following topical treatment of diesel, coal soot or coke oven particle extract at the 20-mg dose.

For all tissues examined, the fraction of the administered dose detected as DNA adducts increased as the dose decreased. This observation was particularly apparent for those skin and lung DNA samples isolated from the coal-soot-exposed (1, 5 and 10 mg) animals (Table III).

Dose–response related differences in DNA adduct levels

DNA adduct levels for all complex mixture exposures were generally higher in skin DNA compared with lung and liver modified DNA respectively (Table II). One exception was noted for the diesel modified lung DNA samples where DNA adduct formation exceeded the number of DNA adducts detected in skin at the 120 mg diesel dose (Table II). The DNA binding capacity of lung from coke-oven- or coal-soot-exposed mice appeared to be maximal at the 20-mg exposure dose. No further increase in adduct labeling was evident at the higher doses. In contrast, lung DNA adduct levels for the diesel-exposed mice increased in a dose-dependent manner over the entire dose range. Liver DNA adduct levels for the coke-oven- and coal-soot-exposed mice also

Table III. DNA-adduct-forming efficiency in mouse skin and lung following topical application of extracts from complex mixtures

Dose	Skin[a]			Lung[a]		
(mg/animal)	Diesel	Coal soot	Coke oven	Diesel	Coal soot	Coke oven
1	ND[b]	38.0	ND	ND	14.6	ND
5	ND	14.7	ND	ND	5.1	ND
10	ND	8.2	ND	ND	3.9	ND
20	0.6	3.8	7.2	0.4	1.6	5.2
50	0.3	1.1	4.2	0.3	0.7	2.2
120	0.2	0.4	1.0	0.3	0.2	1.1

[a]RAL (adducts/10^8 nucleotides) for the diagonal zone of radioactivity divided by the total dose (mg) of extract applied.
[b]Not determined.

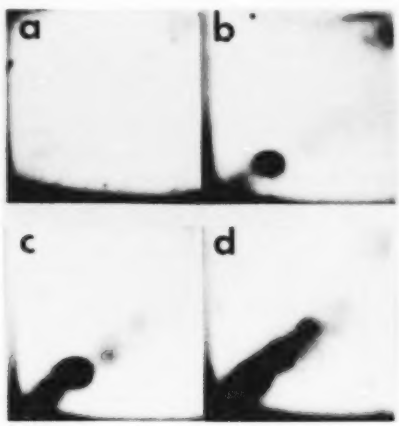

Fig. 4. Autoradiographs of lung DNA isolated from mice topically treated with extracts from coal soot 1 mg/mouse (**b**), 5 mg/mouse (**c**) and 10 mg/mouse (**d**). (**a**) is the acetone control. Autoradiography of the chromatographs was at −80°C for 4 h.

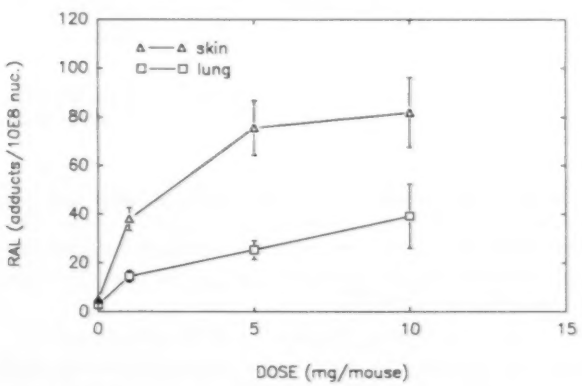

Fig. 5. RAL for skin and lung DNA isolated 24 h following topical treatment of mice with 1, 5 and 10 mg coal soot.

increased in a dose-dependent fashion over the entire dose range (Table II).

To provide additional dose−response information, one additional experiment was conducted with coal soot extract at lower doses (1, 5 and 10 mg). Data from these experiments show a clear dose−response relationship for both skin and lung modified DNA samples. The DNA adduct forming efficiency

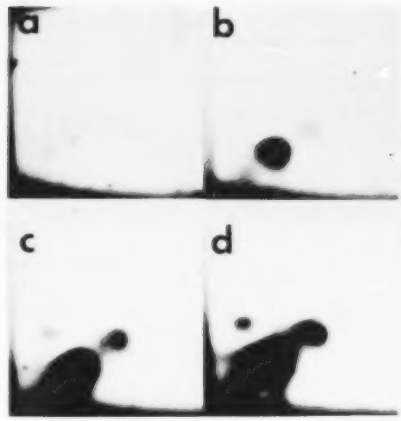

Fig. 6. Autoradiographs of lung DNA isolated from mice topically treated with diesel particulate extract (**a**) acetone control 20 mg/mouse (**b**) 50 mg/mouse (**c**) and 120 mg/mouse (**d**). Autoradiography of the TLC plates was at −80°C for 18 h.

was higher at the lower coal soot doses than would be predicted by a linear extrapolation model (Figures 4 and 5, Table III).

Adduct 1 represents ~20% of the total radioactivity quantitated within the diagonal zone of radioactivity for all three complex-mixture-modified DNA tissues at all doses with the exception of the diesel-modified lung DNA samples where the relative proportion of adduct 1 increased in a dose-dependent manner (Figure 6, Table II).

Discussion

The nuclease P1 version of the standard ^{32}P-postlabeling assay has been shown to detect DNA adducts resulting from the metabolic activation of several structurally diverse chemicals (21). We have examined DNA adduct levels in skin, lung and liver following skin application with 1−120 mg extract from coke oven, diesel or coal soot emissions. The model complex mixtures that were selected have been previously reported to be mutagenic in a variety of short-term bioassays and are tumorigenic in skin tumor initiation studies (11,17). Coke oven emissions also result in increased lung cancer risks (10).

Autoradiographs of the DNA adduct patterns resemble those detected in other complex-mixture-exposed rodent and human tissues (6−8). A diagonal zone of radioactivity, presumably representing multiple putative DNA adducts, was observed for all three complex-mixture-derived DNA samples. Fewer DNA adducts were detected in the diesel-modified skin DNA samples; this finding is consistent with the relatively low levels of PAH present in diesel compared with coke oven emissions (24). DNA adduct levels for the coke oven and coal soot exposures were estimated to be 1.5 times higher in skin compared with lung tissue. These findings are consistent with those of Schoket *et al.* (7) where skin DNA adduct levels, following topical application of bitumen, creosote and coal tar were shown to be ~2-fold higher than those detected in mouse lung. The distribution of DNA adducts reported in our study, contrasts with studies by Randerath *et al.* (6) where cigarette-smoke-condensate-induced injury was higher in lung than skin as measured by the ^{32}P-postlabeling assay. It is not possible to ascertain whether the observed tissue distribution responses are exposure, dose or strain related; however, the latter consideration seems less likely inasmuch as DNA adduct levels were comparable for mouse strains (Swiss mice, DBA/2 and C57BL) following skin

application of three PAHs (25). Our data, taken together with reports in the literature, emphasize that it is difficult to predict the tissue distribution of DNA adducts following exposure to complex mixtures.

Based on co-chromatography experiments one adduct, common to all three complex-mixture-modified DNA samples, was shown to comigrate with a major BaP adduct detected in the BaP-treated rodent tissues. It appears likely that another adduct with similar chromatographic properties compared to the major BaP-derived DNA adduct is formed following exposure of the three particle extracts tested in this study. The relative concentration of BaP within each of these mixtures also suggest that this adduct is not derived from BaP alone. For example, if this adduct is derived from BaP alone then the mice are 10–20 times more efficient in forming this adduct when exposed to diesel compared with coal soot or coke oven particle extract respectively (Table I). The possibility remains, however, that BaP-derived adducts are formed more efficiently when BaP is encountered in diesel extract as compared to coal soot or coke oven. The RAL for this adduct represents ~12–34% of the total number of adducts detected within the diagonal zone of radioactivity for the coke oven and coal soot DNA samples. In contrast, the RAL for this adduct represents 49–67% of the total radioactivity within the diagonal zone of radioactivity for lung DNA isolated from the diesel-treated animals, suggesting that a DNA reactive intermediate formed following diesel-particle-extract treatment is preferentially present in the lung (Table II). These findings are in agreement with studies by Bond *et al.* (8), where a respiratory tract DNA adduct was detected in the approximate region of the TLC plate occupied by a BaP-derived adduct after rats were chronically exposed to diesel emissions. Adduct 1 does not appear to be derived from arylamines or nitroaromatic compounds (22,26). This adduct has also been detected in bacterial DNA following treatment with the identical complex mixtures used in this study (27), which may provide a means for generating sufficient amounts of this adduct needed for chemical identification.

Our treatment and dosing regimens were similar to those employed by Randerath *et al.* (18) in studies conducted to study DNA adduct formation and tissue distribution of DNA adducts in mice dermally treated with cigarette smoke condensate. Cigarette-smoke-induced lung injury as measured by DNA adducts approximated RAL detected in the diesel-modified lung DNA samples, but were lower than the number of adducts detected in lung DNA following coal soot and coke oven exposure. In skin-painting experiments by Schoket *et al.* (7) RAL values for skin DNA isolated for the coal-tar-treated animals were similar to those detected for our coal soot skin DNA samples.

DNA adducts were shown to increase in a dose-dependent manner in lung DNA for all three complex-mixture-treated animals; however, the binding efficiency of the reactive intermediates was shown to be highest in mice treated with the lowest applied doses for all complex mixtures irrespective of tissue examined (Table III). The DNA-adduct-forming efficiency of 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a carcinogenic nicotine metabolite in tobacco smoke, also forms DNA adducts more efficiently at low doses in the lung (28). Our data indicate that more DNA adduction occurs at low doses for the coal-soot-treated mice than would otherwise be predicted by linear extrapolation from high dose. The mechanism for the dose-dependent differences in DNA-adduct-forming efficiency are unclear; however, cell death due to the toxicity of the extract, enzyme inhibition/saturation, DNA repair and/or cell turnover might provide possible explanations.

We have in this study, simultaneously quantitated the overall level of DNA damage resulting from three complex mixtures (exhibiting a wide range of mutagenic or tumorigenic potency) and the formation and distribution of a singular adduct shown to preferentially bind to lung DNA. This is an especially important finding, inasmuch as the lung has been shown to be a target tissue for these and other combustion-related emissions (29). Studies aimed at comparing the relationship between molecular dosimetry and complex environmental mixtures exposures (particularly those with carcinogenic potential) will broaden the data base from which more extensive comparative analyses can be made with complex environmental mixtures.

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